

Genotypic and phenotypic factors contributing to the development and progression of pneumoconiosis

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Chapter 8

Summary and general discussion

Pneumoconiosis induced by inhalation of mineral dusts such as coal dust, silica, and asbestos is still prevalent in industrial countries, and presents an even greater problem in developing nations (Rosenman KD, 1997; Wagner GR, 1997; Banks DE, 1998). Although mineral dusts are the causative agents of pneumoconiosis, only some people similarly exposed to mineral dusts develop pneumoconiosis, and the determinants of susceptibility are poorly understood. Intrinsic factors such as genetic background may be important determinants of interpersonal susceptibility to pneumoconiosis. (Marshall RP, 1997; Nemery B, 2001; Borm PJA, 2001). However, specific genetic factors in the development of pneumoconiosis have not been clearly identified.

In the past years, many new insights on the mechanisms and mediators in mineral dust-induced lung disorders have been obtained from *in vitro* studies, animal studies, and epidemiological studies. Although notable differences exist between different types of mineral dusts, *in vitro* as well as *in vivo* research has yielded considerable similarities in the nature of biological responses, and in spite of their morphological differences, many different dusts share common endpoints including fibrogenesis. Both coal dust and silica particle can stimulate production of oxidants, and cytokines from a number of cell types. Several of these factors may act alone or in concert to cause chemotaxis, cell injury, proliferation, and synthesis of collagen (Mossman BT, 1998; Schins RFP, 1999). On the other hand, it is now evident that polymorphisms have been found in many genes, including cytokine genes and antioxidant genes. These polymorphisms may be associated with altered susceptibility to disease, to disease severity, and to the response to treatment.

In the present thesis, we explored biomarkers for susceptibility to pneumoconiosis on DNA level (genotypes) and protein level (phenotypes). Focusing on cytokines and antioxidants, the two mostly appreciated biological mechanisms involving in the pathogenesis of pneumoconiosis, we studied the representative cytokines (TNF- α , IL-6, TNF- α receptors, IL-6 receptor, and IL-8), oxidant-induced effects (ROS, oxidative DNA damage, DNA strand breaks) and antioxidants (GSTM1,

GST1, MnSOD, OGG1), respectively.

In Chapter 2, we investigated the G-to-A transition polymorphisms at -238 and -308 of the TNF promoter gene in coal miners. Our results showed that frequency of A⁻³⁰⁸ genotype (T2) is significantly overpresented in coal miners with CWP (50%), as compared with miners without CWP (25%) and controls (29%). After correction for cumulative dust exposure and smoking, the A⁻³⁰⁸ genotype is still associated with the presence of CWP (OR=3.0, 95% CI=1.0-9.0). Both A⁻³⁰⁸ and A⁻²³⁸ genotype were related to TNF release from endotoxin-stimulated blood monocytes; only the A⁻²³⁸ genotype and not the A⁻³⁰⁸ genotype was associated with coal dust-induced TNF release. This study shows that A⁻³⁰⁸ genotype is related to CWP development, but this relation is not paralleled by a different TNF release in this genotype.

IL-6 has been implicated in the pathogenesis of silicosis and CWP. A functional G to C polymorphism at position -174 of the promoter of the IL-6 gene has been described. Subjects with the G allele had higher plasma IL-6 levels compared with C allele carriers. To explore the possibility that this IL-6 polymorphism is involved in the pathogenesis of CWP, we examined this polymorphism in a group of coal miners with CWP and compared them with controls consisting of retired coal miners without CWP (Chapter 3). We found that IL-6 polymorphism at -174 is extremely rare in this cohort of Chinese coal miners. Our results suggested that the IL-6 polymorphism at -174 is unlikely to be contributing significantly to disease susceptibility in Chinese populations.

In response to dust-induced ROS, antioxidants such as MnSOD, GSTM1, GSTT1, and OGG1 are induced locally in the lungs and systematically in the blood. The genes encoding for MnSOD, GSTM1, GSTT1, and OGG1 have been found to be polymorphic. These polymorphisms may alter the function of the associated antioxidants and thereby influence the amount of oxidative damage in the development or severity of disease status. To investigate the association between genetic polymorphisms of MnSOD, GSTM1, GSTT1, or OGG1 and susceptibility to CWP, we analyzed allelic frequencies of these genes in 259 ex-coal miners who had documented coal mine dust exposure histories (Chapter 4). The results showed that there were no differences in genotype frequency of MnSOD, GSTM1, GSTT1, and OGG1 between miners with CWP and miners without CWP, by logistic regression analysis. Cumulative dust exposures, but not genetic polymorphisms, were associated significantly with the presence of CWP. This study illustrated the complexity of evaluated genetic factors that may contribute to disease outcome in CWP. The polymorphisms studied may have a limited role in the modulation of ROS-induced damage in CWP development.

In Chapter 5, we investigated whether systemic TNF- α , soluble TNF- α receptors (p55, p75), IL-6 and soluble IL-6 receptor could be markers of biological activities of CWP. Mean serum concentrations of p55, p75, and IL-6 were significantly higher in CWP cases than in controls. Results from logistic regression models showed similar associations between soluble p55 and p75 levels and the presence of CWP. Linear regression analysis revealed that CWP radiographic stage was significantly correlated with individual serum concentrations of p55, p75, and IL-6. Serum levels of all measured cytokines were not correlated to age, dust exposure, or smoking, but there were correlations between p75 and p55, and between p75 and IL-6 levels. The results suggested that serum levels of TNF receptors and IL-6 are associated with the fibrotic process of CWP and serum cytokine levels may be correlated with the severity of CWP.

Although silicosis is widely known to be characterized with a persistent inflammatory response and generation of pro-inflammatory and fibrotic mediators (Mossman and Churg, 1998; Vanhee et al., 1995; Schins and Borm, 1999; Zhai et al., 2002), it is still uncertain which mechanisms are crucial to the processes of inflammation and fibrogenesis in silicosis. Chapter 7 investigated the cellular and cytokine profiles in bronchoalveolar lavage fluids (BALF) of 16 silicosis patients and compared these with bagassosis. We found that the predominant cell in the BALF in silicosis was macrophages while bagassosis was characterized with hypercellularity with neutrophilia in BALF. Compared with control subjects, increased TNF- α , IL-1 β , IL-8, and IL-6 levels were found in the BALFs in both silicosis and bagassosis. Furthermore, IL-6 levels in the BALF of silicosis subjects were significantly higher than that seen in bagassosis. In contrast, bagassosis had higher level of IL-8 in BALF than that in silicosis. Associations were found between IL-8 levels and neutrophils, lymphocytes and IL-1 β in bagassosis, macrophages and IL-1 β in silicosis. No significant differences of total protein concentrations and IL-5 in BALF were found between controls or bagassosis, and silicosis. The findings of this study indicate that even though silicosis and bagassosis share many similarities in inflammation and fibrosis, their cellular and inflammatory cytokine patterns in the airways are different.

It has been shown that the prevalence and severity of CWP differed remarkably between different coalmines despite comparable exposure to respirable dust (Hurley et al, 1982; Attfield and Castellan, 1992; Amoudru, 1987; Reisner and Robock, 1977). The major factors responsible for the observed regional differences in CWP prevalence have not yet been clearly understood. Chapter 8 studied three coal samples with different CWP prevalence for the content of elements, ROS release capacities, and oxidative DNA damage and cytokine production in human lung epithelial

cells. Our data showed that metal compositions differed between these three coal samples. Coal dusts which contained a higher content of iron, generally released more ROS than coal samples with less iron content. Exposure of the human lung epithelial cell line (BEAS-2B) to three coal particles induced different oxidative DNA damage levels and cytokine (TNF- α , IL-6, TGF- β) release rates. Oxidative DNA damage was correlated to CWP prevalence, however, other physicochemical properties of these coal dusts did not associate with the prevalence of CWP. The results of this study indicate that some other mechanisms than metal composition, dust-induced ROS formation, and cytokine production are of more relevance with respect to the regional differences in the prevalence of CWP.

Overall, although the studies presented in this thesis provide a lot of information on genotypic and phenotypic risk factors associating to the development of mineral dust-induced CWP and silicosis, no final conclusion can be drawn regarding the relationship between genotypes, phenotypes and susceptibility to pneumoconiosis. Our studies illustrate the complexity of pathogenesis of pneumoconiosis. As environmental exposure is the primary determining factor in the incidence of pneumoconiosis, the fact that both exposure and the presence of disease can affect the phenotypic marker must be taken into consideration. There is increasing evidence that several genes influence the development of pneumoconiosis. In a complex polygenic disease such as pneumoconiosis it is likely that multiple genes are operating and that the influence of each gene in isolation may be relatively weak. The susceptibility to develop pneumoconiosis, whether CWP or silicosis, is likely to depend on the coincidence of many gene polymorphisms that act together. Pneumoconiosis has several components including abnormal immune response, protease/antiprotease interactions, growth factor metabolism, and impaired collagen turnover (Marshall et al, 1997). There are therefore many possible candidate genes but so far few polymorphisms have been studied in relation to pneumoconiosis.

The effects of gene-environmental interactions on disease are further complicated by both the number of genetic loci that are involved, exposure variables (dose, length exposure), other environmental exposure and the presence of etiologic heterogeneity. The issue of confounders is also a major problem in the assessment of gene-environment interactions. Unmeasured genetic determinants and environmental exposures can each act as confounders. Using new technologies such as gene chip technology it is possible to investigate more than 10,000 gene polymorphisms simultaneously, thus providing insight into the complexity of gene-function relationships by gene expression analysis (Brown and Botstein,

1999). A complementary approach is to search for new genes and proteins by mRNA differential display or proteomics. Identification of genetic and phenotypic markers that predict the prevalence, or that are associated with response to different treatments as they are developed, may help in disease prevention and improved management in the future.